squared displacement (MSD) of single molecule tracks obtained from live cell measurements. Exploiting the photo-switching of PA fluorescent proteins and blinking organic fluorophores, we detect an ensemble of single molecules in each cell investigated, and can analyze populations of diffusers with incredible statistics. By comparing theoretical predictions with quantitative experimental observations, we aim to test our working hypothesis that critical composition fluctuations provide the physical basis of raft heterogeneity.

1. Machta, B.B., S. Papanikolaou, J.P. Sethna, and S.L. Veatch, 2011. *Minimal model of plasma membrane heterogeneity requires coupling cortical actin to criticality.* Biophys J. 100: 1668-77.

423-Pos Board B209

Phosphoinositides Alter Lipid Bilayer Properties

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Phosphoinositides are involved in cell-signaling pathways that regulate vital cell functions such as membrane excitability and trafficking, and cell metabolism, motility and proliferation. At the plasma membrane, phosphatidylinositol-4,5-bisphosphate (PIP_2), which constitutes approximately 0.25% of cell phospholipid, is a key messenger in membrane-delimited signaling. PIP_2 regulates structurally and functionally diverse membrane proteins including voltage- and ligand-gated ion channels, inwardly rectifying ion channels, transporters and receptors. The mechanism(s) by which PIP_2 regulates many of its various ''receptors'' remain to be elucidated. Here we explore the notion that the amphiphilic phosphoinositides, by adsorbing to the bilayer/solution interface, alter bilayer properties such as curvature and elasticity. Such changes in bilayer properties can alter the equilibrium between membrane protein conformational states and thereby alter function. Taking advantage of the gramicidin channels' sensitivity to changes in the lipid bilayer properties, we used fluorescence-based and single-channel gA assays to examine the effects of (diC8) phosphoinositides -PI, $PI(4,5)P_2$, $PI(3,5)P_2$, $PI(3,4)P_2$ $PI(3,4,5)P_3$ as well as long-chain $PI(4,5)P_2$ on the lipid bilayer. The diC8 phosphoinositides, except for $PI(3,5)P_2$, alter lipid bilayer properties with potency that decreases with increasing charge. Among the long-chain $PI(4,5)P_2$s, the naturally occurring 1-stearyl-2-arachidonyl-$PI(4,5)P_2$ is a more potent bilayer modifier than di-oleoyl-$PI(4,5)P_2$. The diC8 and the naturally occurring $PI(4,5)P_2$ have similar effects on short and long gA channels, indicating that changes in bilayer curvature dominate over those on bilayer elasticity. In contrast, diC8PI, which was more bilayer-active than diC8PIP_2 altered bilayer elasticity. Our results show that application of exogenous PIP_2 and its structural analogues (with changes in acyl chain length or phosphorylation state) alters lipid bilayer properties. These PIP_2 lipid bilayer effects may be important for some of the many different effects on membrane protein function.

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Direct Observation of Plasma Membrane Domains using Super Resolution Microscopy

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The composition of the plasma membrane has long been modeled as a mosaic fluid. However, in the last few years there has been evidence that suggests the plasma membrane to be a dynamic and highly compartmentalized structure. This organization in domains results in a differential spatial distribution of signaling proteins on both leaflets of the plasma membrane. It is still debated whether inner and outer leaflet domains are linked. The lateral segregation of membrane proteins plays a role in cell signaling and protein-protein interaction. Thus, it is of high scientific interest to further investigate these domains.

The Ras protein resides on the inner leaflet of the plasma membrane. Here we used super-resolution microscopy to study the compartmentalization of H-Ras and its membrane anchor CAAX, fused to the photo convertible dye Dendra2. The signal of single Dendra2 molecules is recorded and statistical analysis is applied to localize these molecules. On the apical membrane of 3T3 fibroblast, domains of 150nm were detected for both the full protein and its membrane anchor. To investigate a possible link between inner and outer leaflet domains, cells where treated with Choleratoxin B (CtxB). This leads to clustering of the outer-leaflet ganglioside GM1. Neither size nor the amount of domains were dependent on incubation with CtxB. However, incubation with CtxB did lead to an increase in H-Ras density inside the domains, indicating a connection between lipid organization on the outside and protein distribution on the inside of the plasmamembrane.

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Statins Modify Lipid Bilayer Properties

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Statins are drugs that are widely prescribed to manage hypercholesterolemia. Statins exert their primary mechanism of action by inhibiting the HMG-CoA reductase, thus preventing cholesterol synthesis. In addition to this canonical action they also alter the function of diverse membrane proteins. Because statins are amphiphiles that modulate the function of different, structurally unrelated membrane proteins, we investigated whether statins could alter lipid bilayer properties at concentrations where they alter membrane protein function. To this end, we used the gramicidin-based fluorescence assay (GBFA) as well as single-channel electrophysiology. We found that atorvastatin, fluvastatin, lovastatin, mevastatin, pravastatin, and simvastatin all increased the rate of fluorescence quenching, meaning that they shifted the gramicidin (gA) monomer dimer equilibrium toward the formation of conducting dimers. Statins thus alter lipid bilayer properties, with fluvastatin being the most active and rosuvastatin the least active. When examined using single-channel electrophysiology, simvastatin, pravastatin, and fluvastatin increased the lifetime and appearance rate of gA channels with fluvastatin being the most active and pravastain being the least active. We observe larger effects on the shorter channels; the hydrophobic mismatch dependant effects indicate a change in bilayer elasticity. We conclude that statins alter lipid bilayer properties by a common mechanism, through an increase in bilayer elasticity, and that specific channel-statin interactions are not the sole mechanism of action for statins.

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Heterogeneity of Water Dynamics of Hydrated Lipid Bilayers in Atomistic MD Simulations

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Probing dynamics of water molecules interacting with polar headgroups of hydrated lipid membranes is vital in understanding general properties of membrane systems. Recent terahertz spectroscopy experiments provided new insights into dipolar relaxation and dynamics of water molecule reorientation in lipid bilayers with decreasing hydration level[1]. We perform molecular dynamics simulations of DOPC with varied levels of hydration. Our simulation models reproduce the experimental therahertz spectroscopy results with reasonable accuracy. Previously, three different types of water molecules were proposed that were described as irrotational water, bulk water, and fast water with distinct relaxation dynamics. We analyze single molecule dipole correlations in detail to study reorientational dynamics of water molecules in our simulated systems. Our results provide us with distributions of relaxation properties as a function of hydration level. We identify a population of water molecules which are tightly bound to lipid headgroups and exhibit relatively very slow relaxation dynamics. The remaining water molecules in the simulated systems, whose reorientational dynamics can be probed on the timescale of our simulations exhibit a broad heterogenous distribution of dynamical properties. This result suggests that models used to interpret experiments probing the reorientational dynamics of water molecules in a hydrated lipid bilayer should be based on a proper description of this distribution instead of isolated populations of water molecules with distinct properties.

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Understanding Plasma Memebrane Organization and Cellular Homeostasis Relationship

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